Vol. XXXIV. *May, 1918.* *No. 5*

BIOLOGICAL BULLETIN

SPERMATOGENESIS OF THE PACIFIC COAST EDIBLE CRAB, *CANCER MAGISTER* DANA.

NATHAN FASTEN,

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF WASHINGTON, SEATTLE.

CONTENTS.

1. INTRODUCTORY REMARKS.

The Decapoda have always been of absorbing interest to the cytologist and considerable has been written concerning their germ cells. In a previous publication ('14), I have given a review of the important literature on the subject, and by referring to this article it becomes apparent that practically all the investigations have been confined to the male reproductive elements of the Macrura and Anomura. Virtually nothing has been done with the Brachyura; in fact there isn't a single complete spermatogenesis of a crab known. Carnoy ('85) observed and pictured a few of the early proliferation stages in the sper-

matogenesis of *Carcinus menas*, while Binford ('13) hastily described the spermatogenesis of the Atlantic coast edible crab, *Menippe mercenaria*. None of these investigators, however, have given details regarding the interesting processes of synapsis, reduction, chromosome numbers and the like.

During the past few years the writer has been gathering testicular material of numerous crabs which occur along Puget Sound, for the purpose of studying their spermatogenesis, and in the following pages the spermatogenesis of one of these forms, namely, *Cancer magister*, is described.

2. Material and Methods.

As already mentioned, the material from which these studies were made consisted of the testis of the common edible crab of the Pacific coast, *Cancer magister*. This Brachyuran is widely distributed along Puget Sound, and an abundance of material was available for study. Most of this was gathered in the vicinity of the Puget Sound Biological Station, Friday Harbor, Washington, during the summers of 1915 and 1916. In the latter part of June and the early portion of July, the testis of *Cancer magister* is in the best condition for the study of spermatogenesis. At these times every stage in the spermatogenesis process may be found within the male gonads.

The author worked almost exclusively with smears of the testicular cells. Sectioned material was also used for comparison and checking up results. The smears, however, were of greatest service and virtually all the deductions and illustrations were made from them. The manner in which the smears were prepared was as follows. Small pieces of the fresh testis were quickly mashed between two slides and immediately fixed in Bouin's fluid for about ten minutes. The slides were then washed in water until all traces of the yellowish picric acid were removed. They were next stained by the iron-alum haematoxylin method, with a counterstain of acid-fuchsin. Finally, they were run up in the usual manner through the alcohols into xylol and when fully cleared were mounted under cover-glasses with Canada balsam. Some of these preparations were unsurpassed for details regarding synapsis, chromosome numbers,

spermatid transformations, and steps in the opening of the spermatozoa. With the exception of Figs. 1–15 and Figs. 67–69, all the drawings in the accompanying plates are from smear preparations. Figs. 1–15 are from sections, while Figs. 67–69 are from living spermatozoa as viewed in the crab's body fluids.

The material for sectioning was cut into small pieces and fixed with numerous fluids. The best fixatives, however, were found to be Flemming's strong and the Meves-Duesberg modified Flemming. The sections were cut 5μ in thickness and then stained by either the iron-alum hæmatoxylin method with a counterstain of acid-fuchsin or by the safranin, gentian-violet method.

The living spermatozoa were studied under the oil-immersion lenses in various fluids, such as the body fluids of the crab, sea water, in isotonic and hypotonic solutions of various salts (NaCl, KCl, $CaCl_2$, $NaNO_3$ and KNO_3), and in distilled water. By fixing the spermatozoa on the slide with either osmic acid fumes or Bouin's fluid, these structures could then be stained in iron-hæmatoxylin and acid-fuchsin and all the stages in their explosion could subsequently be studied.

3. Description of Testis.

The testis of *Cancer magister* has already been described elsewhere (Fasten, '15). It is a bilobed organ lying in the cephalothoracic region, below the cardiac chamber and above the digestive glands. Each lobe is profusely tubular and runs laterally along the stomach. During June and July these testicular lobes are prominently developed and fill up a large part of the upper cavity of the cephalothorax. Below the anterior portion of the heart the two lobes of the testis unite, and from this junction point two stoutly convoluted tubes, the vasa deferentia, originate and run posteriorly to the base of the fifth pair of walking legs where they open to the outside.

During the latter part of June and the early part of July the tubules in the outer and middle regions of each testicular lobe are usually undergoing rapid proliferation. Some of them are filled with spermatogonia, while others contain growth stages and still others show primary and secondary spermatocyte

divisions. Also, many of the tubules show spermatids transforming into spermatozoa, although the mature spermatozoa are mainly confined to the tubules located near the inner or median portion of the testicular lobe as well as in the spermatophores of the vasa deferentia.

When sections of the tubules are examined, many of them are observed to be constructed on the same plan as those of *Menippe mercenaria* which were described and pictured by Binford ('13). Two or three well-defined zones may oftentimes be distinguished in one tubule. For instance, at one pole in the sectioned tubule there might be a thin layer of spermatogonia and the rest of the tubule might contain either transforming spermatids, or mature spermatozoa, or primary spermatocyte divisions, or those of the secondary spermatocyte stage, or even growth stages. When there are three zones in the tubule these are often made up of (1) a thin region of spermatogonia at one end, (2) a middle portion filled with transforming spermatids, and (3) a region of mature spermatozoa filling in the opposite pole.

The mature spermatozoa, when they enter the vasa deferentia, are surrounded by pouches known as spermatophores (Fig. 67). These spermatophores are formed by the secretions of the inner layer of epithelium which lines the vas deferent tube. For a more detailed description of these structures see Fasten, '17. During copulation the spermatophores are discharged from the vasa deferentia of the male and are deposited within the seminal receptacle of the female, where they remain dormant until the ova are mature for fertilization.

4. Spermatogenesis.

A. *Spermatogonial Stages.*

The spermatogonial cells generally line one end of the tubule. They are fairly large cells, with distinct cytoplasmic outlines and prominent nuclei. In *Cancer magister*, primary and secondary spermatogonial stages can be distinguished (Figs. 1–8). Of the two, the primary spermatogonium (Fig. 1) is somewhat larger in size, and from it by division (Figs. 2–7), is derived the secondary spermatogonium (Fig. 8).

The resting primary spermatogonium (Fig. 1) contains numer-

ous large chromatin clumps within the nucleus. In some instances a number of linin strands could also be distinguished. The cytoplasm is more or less uniform throughout, but in a few cases large heavily staining masses, surrounded by clear outlines (Fig. 2), could be observed within it. A centrosome is almost always discernible.

When the cell begins to divide the large chromatin clumps of the nucleus undergo fragmentation (Fig. 2). This process continues until the chromatin becomes organized into a great number of heavily staining elliptical or oval structures distributed throughout the nucleus. The spermatogonium at this stage presents the picture shown in Fig. 3. Counts of these chromatin clumps were attempted, but they were found to vary considerably, ranging everywhere from forty to sixty-five. Binford ('13) has made similar observations in *Menippe mercenaria* and in the American crayfish *Cambarus virilis*, I ('14) have found the same sequence of events. Binford regards these chromatin clumps as the chromosomes which enter the equatorial plate of the spermatogonial metaphase. While these through a further fragmentation, undoubtedly form the ultimate number of chromosomes found during the metaphase stage of the spermatogonial division, yet I am inclined to the view that many of these chromatin clumps represent more than single chromosomes. This conclusion was arrived at after examination of numerous polar views of the chromosomes in metaphase plates of primary spermatogonia, in which over one hundred chromosomes could be counted. Furthermore, these chromosomes were smaller than the chromatin clumps shown in Fig. 3.

The nuclear wall surrounding the chromatin clumps soon breaks down and the cell enters the metaphase stage (Fig. 4). The chromosomes in the equatorial plate are dumb-bell shaped and the spindle fibers are rather delicate in appearance. The centrosomes can be easily distinguished at opposite poles. In polar views of sections of the equatorial plate, the chromosomes are observed to be distributed throughout the whole plane of the equator. They are small and spherical in appearance, and are so numerous that their exact number could not be determined. As already mentioned above, in many instances over one hundred

of them could be counted. But on the assumption that there are twice the number of spermatogonial chromosomes as are found in the reduction division, there must be one hundred and twenty chromosomes in the spermatogonial stages of *Cancer magister*.

The anaphase (Fig. 5), and telephase (Figs. 6 and 7) stages follow each other quickly, thus dividing the cell into secondary spermatogonia (Fig. 8). These are somewhat smaller than the primary spermatogonia (compare Figs. 1 and 8), and in the resting condition (Fig. 8), the chromatin of their nuclei stains much more heavily. Otherwise, the secondary spermatogonia resemble the primary ones markedly and their division proceeds in exactly the same fashion. The ultimate divisions of the secondary spermatogonia produce the resting primary spermatocytes (Fig. 16).

In many spermatogonial strips of the tubules, some of the cells were found to be undergoing degeneration. In such cases, the cells lose their distinctness of outline and their nuclei come to lie in a syncitial mass of protoplasm. In many instances, the nuclei resemble those of the spermatogonia (compare Fig. 9 with Fig. 1); in others (Figs. 10–12), the nuclei become transformed into very large irregular structures with pseudopodia-like projections. These cells are the so-called "nutritive cells" and they may be best studied in tubules where mature spermatozoa are found. In *Cambarus virilis*, I ('14) have noticed similar cells.

The nutritive cells (Figs. 9–15) are very interesting structures. Their nuclei contain heavily staining chromatin masses, while the cytoplasm possesses numerous fat globules which stain intensely black with osmic acid. Many of the earlier investigators on the Decapoda such as Grobben ('78), Gilson ('86), and Herrmann ('90) have claimed that the spermatogonia are derived from the nutritive cells. On the other hand, St. George ('92), and Keppen ('06) claim the opposite, that the nutritive cells are derived from a transformation of the spermatogonia. This last-mentioned condition seems to be the case in *Cancer magister*.

When sections of the nutritive cells are examined, numerous stages like those seen in Figs. 13–15 may be observed, which

strongly suggest amitosis. The fact is that the earlier workers on the Decapoda all claim such division in the nutritive cells. However, a careful study of the nuclei shows many of them to be amboeoid in appearance (Figs. 10–12), and this being the case, it is entirely possible to derive the stages represented in Figs. 13–15 from sections through such cells as are shown in Figs. 10–12. It is, therefore, very difficult as well as dangerous to come to any certain conclusions concerning amitosis from sectioned material.

B. *Primary Spermatocyte Stage.*

This stage follows the spermatogonial divisions. After a period of growth and synapsis, reduction occurs. During the growth period two definite spherical bodies, surrounded by clear spaces, make their appearance in the cytoplasm. These are the so-called chromatoid bodies, and they appear to be similar to the same structures which I ('14) have previously described in *Cambarus virilis*.

(*a*) *Growth Period.*—This period includes the preparatory stages, synapsis and tetrad formation. During the early prophases the chromatin in the nucleus of the resting primary spermatocyte (Fig. 16), consists first of a few large, heavily staining clumps, but these soon undergo a fragmentation process, whereby smaller chromatin masses (Fig. 17) are produced. In a few cells, linin threads which were rather indistinct and granular in appearance were observed. Sometimes large chromatoidal masses like those shown in Fig. 17 were seen within the cytoplasm.

The chromatin of the nucleus breaks up into still smaller structures and these then begin to weave out into thin leptotene threads (Fig. 18). At the same time the cell increases somewhat in size (compare Figs. 16 and 17 with Fig. 18). This stage, represented by Fig. 18, really marks the beginning of the growth period and from now on the increase in the size of the cells is more evident. When Fig. 18 is examined it can be seen that no continuous spireme is formed. The leptotene threads remain separate and may be distinguished from each other. Owing to the great number of these threads it was impossible to count them.

The primary spermatocytes now undergo synizesis. The leptotene threads migrate to one pole of the nucleus (Fig. 19),

the so-called "synaptic pole" and arrange themselves in parallel pairs. This paired arrangement is very distinct and has been observed in a great many cells. While this is going on, two large round bodies, of more or less equal size, and surrounded by clear spaces (Fig. 19, *k*) make their appearance in the cytoplasm. These bodies were first observed in a smear preparation which was heavily stained. They stained exactly like the chromatin. In preparations which were strongly destained no trace of them was found. However, when these latter preparations were restained, the bodies loomed up with exceptional clearness. It is thus seen that although these structures stain like chromatin, yet they differ from chromatin in their affinity for nuclear dyes, and, in all probability, they are chemically different from chromatin. Wilson ('13), first described a similar body in *Pentatoma*, and called it a "chromatoid body." In *Cambarus virilis*, I ('14) have found a pair of chromatoid bodies appearing at about the same stage in the developing spermatocyte as in *Cancer magister*. After the leptotene stage, the chromatoid bodies of *Cancer magister* persist within the cells, and their subsequent history will be outlined when the respective stages of the maturation are dealt with.

Synapsis soon sets in. The parallel threads become more closely paired at the synaptic pole and the cell enters the pachytene stage, in which the pairs of leptotene threads fuse into thick gemini (Fig. 20). Here the union appears complete as no traces could be found of the longitudinal furrows which separated the original pairs of parallel threads.

After remaining fused for some time, the components of each geminus begin to unravel. Along every geminus a longitudinal split makes its appearance (Fig. 21), and simultaneously with this there is an opening of the paired threads at one end, while remaining attached at the other end, thereby producing figures which appear like 8, V, or less commonly like U (Figs. 21 and 22).

Another longitudinal split soon occurs along the arms of each geminus. In Fig. 22 the beginnings of this second longitudinal furrow are clearly visible in the two arms of the 8 which is located in about the center of the nucleus. By means of these two longitudinal cleavage planes, four chromatin threads are formed,

distinctly opened up at one end and temporarily fused at the opposite end. The two pairs of these threads continue to diverge in opposite directions until they ultimately form X's (Fig. 23).

The pairs of threads on opposite sides of the central fusion point of each X now migrate closer to each other until they come to lie almost parallel. The central fusion point next disappears, and four thin threads arranged in two parallel rows are produced. A hasty glance at this arrangement makes it appear as if the geminus from which the four threads were derived, was traversed by a longitudnal and a transverse cleavage plane (see Fig. 23). However, prolonged and careful study reveals the true nature of the case, that the threads under discussion are the results of two longitudnal splits of each geminus.

The tetrads are soon formed. Each thin thread shortens and thickens into a spherical chromatin mass (Figs. 23 and 24), so that very soon every geminus is changed into four spherical chromosomes, representing a tetrad (Figs. 23–25). Each tetrad thus contains four univalent chromosomes. In the next step pairs of these univalent chromosomes fuse, resulting in two large bivalents attached to each other by linin strands. This condition is particularly well shown in Figs. 24 and 25. The condensation of the bivalents continues until they are changed into dumb-bell shaped structures (Fig. 25). The growth process is now completed and the cell is ready to undergo reduction.

It is quite evident from the above discussion that in *Cancer magister* we apparently have to deal with a case of parasynapsis, or side by side conjugation of the chromosomes. This conclusion was reached after prolonged observations upon numerous excellent smear preparations, which contained an abundance of synapsis material. The essential steps closely resemble those of *Cambarus virilis* (Fasten, '14).

(*b*) *Reduction Division.*—In the last stages of the growth period, the nuclear wall begins to break down (Fig. 24). The two centrosomes, which have been formed by a division of the original centrosome, migrate to opposite poles, and thin spindle fibers make their appearance between them and the chromosomes. The chromosomes are soon pulled to the equator of the cell to undergo reduction. In Fig. 25, the dumb-bell shaped bivalents

are seen arranging themselves. Here the chromatoid bodies can also be clearly discerned at opposite poles. Each of these bodies is surrounded by its characteristic clear space and may be easily recognized from the ordinary chromosomes.

In the metaphase period (Fig. 26), all of the chromosomes are grouped in the equatorial plane and appear as large dumb-bells. When the component bivalents of each dumb-bell are examined, they are found to be large spherical bodies, about twice the size of the spermatogonial chromosomes. Furthermore, these bivalents do not show the equational furrows where the equational division of the second spermatocyte will take place. Binford ('13), in describing this stage in *Menippe mercenaria* claims that the chromosomes in the equatorial plate of one of his preparations were found to be ring-like in appearance. In other preparations which were destained to the degree of removing all the stain from the cytoplasm and the achromatic figure of the metaphase, this same investigator asserts that he could distinguish the individual four chromosomes of each tetrad. In *Cancer magister* no such results were obtained in spite of the fact that a great many smears and sections were carefully examined.

In polar views of the metaphase stage sixty chromosomes have been distinguished (Figs. 27 and 28). These are generally oval in shape; some of them being larger than others and they are distributed throughout the equatorial plane. Figures 27 and 28 are drawings of polar views as seen in smear preparations. Similar counts of polar views in sectioned material have corroborated this number for the reduction division.

The chromatoid bodies always migrate undivided to opposite poles of the cell. In many cases they occupy positions along the spindle fibers (Figs. 26, 29 and 30), while in other cases they may be seen in the cytoplasm (Figs. 31 and 32). When they occupy positions along the spindle fibers, one would be easily misled into regarding them as accessory chromosomes, especially so if no attention were paid to the various stages of the growth period. Wilson ('13) has cited numerous cases in which investigators have undoubtedly confused chromatoid-like bodies with accessory chromosomes. After citing these instances, Wilson, on p. 403, then makes the following significant remarks:

"Such facts make it clear that the presence of sex-chromosomes can not safely be inferred alone from the presence of chromosome-like bodies lagging on the spermatocyte-spindles, or lying near one pole. The presence of compact, deeply staining nucleoli during the growth-period is by itself equally indecisive. In some cases the 'plasmosome,' especially after certain fixatives such as Bouin's fluid, may stain quite as intensely as the chromosome-nucleoli with haematoxylin, safranin and other dyes (cf. Gutherz, '12). Decisive evidence regarding these bodies can only be obtained by tracing their individual history and by accurate correlation of the chromosome-numbers in the spermatogonial and spermatocyte-divisions. It hardly need be added that great caution is necessary in dealing with difficult material in which for any reason such a test cannot be completely carried out."

The anaphase stage (Fig. 29) follows upon the metaphase. The bivalents of the dumb-bells are separated and pulled to opposite poles. The chromatoid bodies also migrate in these directions. The division process continues and gradually the primary spermatocyte enters the telophase stage (Figs. 30 and 31). In the final telophase (Fig. 32), the chromosomes which have completely migrated to opposite poles become surrounded by thin nuclear walls. The cytoplasm has constricted off into two distinct portions and during this process the spindle fibers have also been constricted so that at their center a thickened "zwischenkörper" is formed. When this stage is completed two secondary spermatocytes are produced, each possessing one of the chromatoid bodies within the cytoplasm (Fig. 32).

C. *Secondary Spermatocyte Stage.*

The secondary spermatocytes formed during the ultimate telophase stage of the reduction division, undergo immediate transformations. No rest period could be determined. This is similar to what occurs in *Astacus fluviatilis* (Prowazek, '02), in *Menippe mercenaria* (Binford, '13), and in *Cambarus virilis* (Fasten, '14). The cells assume the metaphase stage (Fig. 33), and the chromosomes line up in the equator of the spindles in the form of dumb-bells. When polar views of these chromosomes are studied in preparations which have been greatly destained,

sixty of them may be counted (Figs. 34 and 35). These chromosomes are about half the size of those found in the reduction division (compare Figs. 34 and 35 with Figs. 27 and 28). As for the chromatoid body, it generally lies at or near one pole of the cell, leaving the other pole without any such element.

The anaphase (Fig. 37) and telophase (Figs. 38 and 39) stages follow each other in logical sequence, resulting in the division of the secondary spermatocytes to form spermatids. Two types of spermatids (Fig. 40), are thus formed in equal numbers; one type which contains a single chromatoid body in the cytoplasm (Figs. 40 and 41), whereas, the second type is without this body (Figs. 40 and 43).

D. *Transformations of the Spermatids into Spermatozoa.*

The spermatids produced are, at first, small and their nuclei contain large masses of chromatin material which stain intensely with nuclear dyes (Fig. 40). The cytoplasm is homogeneous throughout and within it a rather prominent centrosome is found. In the second type of spermatid developed, the cytoplasm, in addition to containing the centrosome, also possesses the chromatoid body (Figs. 40 and 41).

The first noticeable changes undergone by the spermatids in transforming into spermatozoa, occur in the nucleus. The chromatin mass of the nucleus is gradually reduced to such a degree that it loses its intense staining qualities, and becomes quite homogeneous in consistency (Figs. 40–47). At first the large chromatin clumps break up into granular masses (Figs. 40, 43 and 44). Then these fragment still more completely until in the final stages there remain, respectively, three round chromatin bodies (Fig. 45); then two (Figs. 42 and 46), and ultimately one (Fig. 47). This remaining chromatin structure is spherical, stains intensely black with Heidenhain's haematoxylin and occupies the center of the nucleus. It may be said to be a nucleolus-like body which resembles a karyosome.

Both classes of spermatids produced undergo similar changes of the nucleus. In the second type of spermatid, however, an interesting change goes on in the cytoplasm, simultaneously with the transformations of the nucleus. Here the chromatoid body

wanders from its position within the cytoplasm to the periphery of the cell and is soon expelled to the outside (Figs. 40–42), thus playing no further rôle in the transformations. From now on all the spermatids are exactly alike and they undergo the same modifications.

At about this time a densely staining mass makes its appearance in the cytoplasm (Figs. 46 and 47, *m*). This mass has been called a mitochondrial mass by Koltzoff ('06) and Binford ('13). It stains like the chromatin of the nucleus, and first makes its appearance at about the stage in the spermatid transformations where the chromatin of the nucleus becomes reduced to two clumps (Fig. 46). As to whether this mass consists of mitochondria or not is a debated question. The fact of the matter is that cytologists themselves are not clear as to which bodies within the cell are mitochondria and which are not (see Cowdry, '16). In the cells under consideration, no traces of mitochondria have been observed in the earlier stages of the maturation. This darkly-staining mass makes its appearance only after most of the chromatin within the spermatid nucleus has been much reduced. This would lead one to suspect that the mass might consist of chromatin which has diffused out of the nucleus and has accumulated within the cytoplasm (see Figs. 43–47). At any rate, this seems a likely probability.

The spermatids now reveal the following distinct structures, (1) a rather homogeneous nucleus (Fig. 47, *n*), with a karyosome-like body in the center, (2) a cytoplasm in which are found (3) a centrosome (Fig. 47, *c*), and (4) a mitochondria-like mass (Fig. 47, *m*), which stains like chromatin. These elements must be clearly kept in mind in order to follow up the later changes of the spermatids. In linking up these changes it is also necessary to exercise great care. Sectioned material, while helpful, is by itself wholly inadequate for this purpose. Smear preparations of the entire cells, on the other hand, give a true picture of what happens, and these were used almost entirely in the study of transformations.

The nucleus wanders to one pole of the spermatid (Figs. 48–50, *n*), while at the opposite pole a clear vacuole (Fig. 50, *v*),

makes its appearance. Sometimes two clear openings (Figs. 48–49, *v*) may be seen, but these later flow together into a single one (Fig. 50, *v*). At the same time the mitochondrial mass wanders in between the nucleus and the vacuole (Figs. 48–52, *m*), and ultimately fills this entire space (Fig. 53). The centrosome increases somewhat in size and takes a position in the center of the mitochondrial mass (Figs. 50–53, *c*).

The mitochondrial mass now transforms into a ring resembling a doughnut, and the centrosome comes to occupy the center of its inner open space (Figs. 53 and 54, *c*). The upper portion of the nucleus also becomes located in this space (Figs. 54–58, *n*). At the same time, the karyosome-like body, situated within the center of the nucleus (Figs. 47–53) migrates upward to the middle of the upper portion of the nucleus (Fig. 54) until it comes to lie directly below the centrosome.

Binford ('13) in describing the transformation of the spermatids of *Menippe mercenaria* claims, on p. 156, that "after the mitochondrial ring is completed the nucleus becomes widely separated from it and the capsule (Figs. 50 to 52). This, however, is not always the case. In two preparations from which Figs. 33 to 35 and 37 to 43 were drawn, the nucleus remained fitted closely on the capsule as shown in Fig. 43. As the two different conditions were obtained with the same fixing fluid it is hardly probable that the difference was caused by the fixing."—In sections of testicular material of *Cancer magister*, a few of the transforming spermatids showed the conditions which Binford describes, but in smear preparations not a single such instance was discernible. I therefore suspect that Binford had to deal with defects which are often produced in cytological material which is prepared by the fixation and the sectioning methods.

Simultaneously with the last-mentioned changes, a second vacuole (Fig. 54, *v'*), makes its appearance in the anterior extremity of the original first (Fig. 54, *v*), or primary vacuole. At first this second vacuole looks like a small bubble of liquid which stains rather darkly with Heidenhain's haematoxylin. It soon increases in size, becomes more distinct (Figs. 54–57, *v'*), and stains somewhat lighter. It is quite evident that Binford's ('13) so-called "inner tubule" formed during the spermatid

transformations of *Menippe mercenaria*, is the same structure which I have called the second vacuole in *Cancer magister*. My preparations, however, show no such stages which Binford describes and pictures (Figs. 52–60 of Binford, '13), for the development of this structure.

During this time the centrosome and the karyosome-like body of the nucleus unite (Figs. 54–56), and elongate into a rod-like structure (Figs. 57 and 58), the so-called central body (Figs. 56 and 57, *b*). At first the central body looks like a dumb-bell (Figs. 56 and 57, *b*), but as it lengthens out, it loses this appearance, becomes more rod-like (Figs. 58–60), and at the same time penetrates the inner or proximal portion of the second vacuole. While all these changes are going on the primary and secondary vacuoles are gradually transforming into primary and secondary vesicles (see Figs. 54–60, *v* and *v'*), and from now on they will be designated by the latter names.

At about the stage represented by Fig. 59, an opening makes its appearance in the middle of the outer, or distal end of the second vesicle. Simultaneously with this, the central body elongates still more (Fig. 59) and its outer extremity seems to hollow out into a thin tube (Figs. 60–62) which soon connects up with the distal opening in the secondary vesicle. As the outer end of the central body hollows out, a ring of densely staining material makes its appearance around the outer opening of the second vesicle (Figs. 61–63, *d*). In Heidenhain's haematoxylin this ring stains intensely black like the centrosome or the chromatin. This ring may be spoken of as a chromatin-ring and it becomes more distinct as the distal end of the central body hollows out (Figs. 61–64, *d*). Going hand in hand with these modifications are those which take place in the mitochondria-like ring and the nucleus. These two elements fuse into a single structure, a sort of nuclear-mitochondrial cup (Fig. 62, *h*), which stains quite homogeneously, although some part of the mitochondrial mass seems to persist around the wall of the first vesicle and stains more intensely than the rest of the cup. (see Fig. 62, *h*).

As the transformations continue, the second vesicle fits more compactly into the first vesicle (Figs. 63 and 64). The outer portion of the central body has completely changed into a thin-

walled hollow tube, which stains lightly. The inner portion of the central body is solid, stains intensely with nuclear dyes and is embedded in the center of the nuclear-mitochondrial cup, immediately below the wall of the first vesicle. Figs. 63–65 show these structures nicely. Now the radial arms or rays of the spermatozoön (Figs. 63 and 64, *r*) make their appearance. They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities (Figs. 63 and 64, *r*).

Soon the spermatids are completely transformed into mature spermatozoa, and in this state (Figs. 65 and 66) they look like ovoid, or spheroid structures, in which the radial rays are tightly coiled around the nuclear-mitochondrial cup. Figs. 65 and 66 are drawings of mature spermatozoa as viewed, respectively, from the side and bottom. An examination of these figures shows the central body (*b*) located in the middle, and surrounding it in order of sequence are the second vesicle (*v'*), the first vesicle (*v*) and the nuclear-mitochondrial cup (*h*). In Fig. 65, the structure of the central body (*b*) consisting of a hollow distal end and a solid proximal end, can be distinctly observed. In this figure may be also seen the densely staining chromatin-ring (*d*), located at the upper end of the secondary vesicle.

When the mature spermatozoa are studied in smear preparations which have been fixed with Bouin's fluid and stained by the iron-haematoxylin and acid-fuschin methods, then the second vesicle stains a dark amber color, while the primary vesicle takes almost no stain and remains clear. In sections fixed with Flemming's fluid and stained similarly, the second vesicle remains more or less transparent, while the first vesicle stains a dark brown.

The mature spermatozoa are at first free, but when they make their way into the vasa deferentia they are surrounded by the membranous pouches or spermatophores already mentioned under the discussion of the testis. Fig. 67 shows one of these spermatophores when it is first removed from the vas deferens of the living crab. Notice the numerous spermatozoa which are compactly stored within its interior.

5. Opening of the Mature Spermatozoa.

The mature spermatozoa within the spermatophores are dormant structures, but when they are removed from the spermatophores and placed in fluids whose concentration is less than sea water, they undergo interesting changes. In many ways these are similar to the changes which Binford ('13) describes for the spermatozoa of *Menippe mercenaria.*

The method employed in studying the opening of the mature spermatozoa of *Cancer magister* was similar to that employed by Binford ('13). Numerous spermatophores suspended in either crab's body fluids, or sea water, were placed on a slide and covered with a cover-glass. By applying pressure to the cover-glass many of the spermatophores were ruptured, thus liberating the spermatozoa. These could then be examined under the high powers of the microscope. By allowing numerous salt solutions, already mentioned under the section on 'Materials and Methods,' to diffuse under the cover-glass, all of the changes in the opening up process or the so-called explosion of the spermatozoa could be followed out quite accurately. Many of these spermatozoa in various stages of their explosion were fixed and stained on the slides and were then used for later study and comparison.

In the crab's fluids, in sea water, or in solutions of NaCl, KCl, $CaCl_2$, $NaNO_3$ and KNO_3 which are isotonic with sea water, almost no change occurs. Usually the rays of the nuclear-mitochondrial cup unravel, revealing spermatozoa which contain either three (Fig. 68) or four (Fig. 69) rays. In hypotonic solutions of the last-mentioned salts, the spermatozoa undergo an explosion, and change considerably in appearance. Osmotic pressure, undoubtedly, accounts for this explosion as was suggested by Koltzoff ('06).

The first step in the explosion of the spermatozoa is the extrusion of the second vesicle. This vesicle normally surrounds the central body and is embedded in the first vesicle (Figs. 70 and 71). When the second vesicle begins to extrude, it swells somewhat (Figs. 72–76, *v'*) in size and at the same time it stretches the upper portion of the first vesicle and makes it appear like a thickened ring (Figs. 72–76, *s*). Simultaneously with this, the

hollow distal end of the central body which is also extruded, exerts a pull on its solid proximal portion, transforming it into a spine-like structure (Figs. 72–76, *b*), which stains intensely black with Heidenhain's hæmatoxylin. While these changes are going on, the nuclear-mitochondrial cup loses its rays and rounds out into a spherical body.

When the second vesicle has been completely extruded (Fig. 75, *v'*), then the first vesicle (Fig. 75, *v*) commences to evert and continues this process until it is completely turned inside out. These steps may be observed in Figs. 75–79, *v*. During the eversion, the darkly staining proximal end of the central body forces upward on its tubular distal portion until the latter is finally extruded completely to the outside (see Figs. 75–77, *b*).

The completely exploded spermatozoa present the appearances represented in Figs. 78 and 79. The upper portion consists of the second vesicle (*v'*), in the interior of which is contained the everted first vesicle (*v*), with its upwardly projecting spine-like body (*b*). The lower portion consists of the nucleo-mitochondrial cup (*h*), which has transformed into a more or less spherical structure. In many cases, stages like those shown in Fig. 80 were seen. These evidently are exploded spermatozoa in which the second vesicle has completely ruptured and disintegrated. Binford ('13) has observed similar conditions in *Menippe mercenaria*.

6. Discussion.

A. *Synapsis.*

During the last few years the parasynaptic view of chromosomal conjugation has been established in numerous species of animals. In 1900, Von Winniwarter first advocated parasynapsis amongst the mammals, but the view did not become firmly established until the Schreiners ('04, '05, '06, '07 and '08) published their important researches on the germ cells of many animals including mammals, birds, reptiles, amphibians, fish, molluscs and annelids. Of late the parsaynapsis view of Von Winniwarter and the Schreiners has been extended to a great many additional forms, and excellent reviews of the vast literature on this subject may be found in the recent publications of Montgomery ('11), Wilson ('12), Fasten ('14) and Wenrich ('16).

Among the Crustacea, parasynapsis has been established in some of the Copepoda (Lerat, '05; Matschek, '09; McClendon, '10; and Kornhouser, '15), and also in one of the Decapoda (Fasten, '14). In the decapod crustacean *Cambarus virilis*, I ('14) showed that during the growth period, the chromosomes conjugate in parasynaptic fashion, and as already pointed out elsewhere in this paper, this is the type of conjugation which occurs in *Cancer magister*. In both *Cancer magister* and *Cambaras virilis*, the great difficulty encountered in the study of synapsis was the immense number of chromosomes. However, after prolonged and careful study of the various stages in the growth period of these animals, one finds it rather difficult to interpret the conjugation of the chromosomes in any other way than by parasynapsis.

B. *The Chromatoid Bodies.*

Ever since Wilson ('13) called attention to a chromatoid body in the spermatogenesis of *Pentatoma*, similar structures have been described during the spermatogenesis stages of other forms. In *Cambarus virilis* (Fasten, '14), two such bodies were found which could be traced into the spermatid stages and then all traces of them were lost. In *Cancer magister*, a pair of chromatoid bodies make their appearance during the synizesis stage of the growth period, and during the reduction division these pass to opposite poles, so that the secondary spermatocytes each possess a chromatoid body. During the equation division, this body passes undivided to one pole, resulting in two types of spermatids, one type possessing a chromatoid body, while the other type is minus such a structure. It is also of interest that the chromatoid body is eventually expelled from the first type of spermatid thus playing no further part in spermatogenesis.

Concerning the nature and function of the chromatoid body, very little can be said. In only two forms, namely *Pentatoma*, (Wilson, '13) and in the decapod under consideration *Cancer magister*, has the full history of this structure been traced, and in both cases, it is expelled from the spermatids, thus appearing to play no definite rôle in the mature spermatozoa. Wilson ('13), in discussing the chromatoid body, makes the following

trite remarks on page 402: "The nature of the chromatoid body thus remains problematical, but the facts are worthy of serious attention for another reason. Were the chromosomes very small, numerous, closely crowded, or otherwise unfavorable for exact study, and could not the entire history of the chromatoid body be so clearly traced, even an experienced observer might fall into the most confusing error concerning the relations of the chromosomes."

7. Summary.

1.. During the latter part of June and the early part of July the testicular lobes of *Cancer magister* are in the best shape for the study of spermatogenesis.

2. Two spermatogonial divisions can be recognized, and these ultimately form the resting primary spermatocytes.

3. Sometimes, larger and more intensely staining cells are found interspersed among the spermatogonia. These are the nutritive cells, and it seems very probable that they have originated from a transformation of some of the spermatogonia. The nuclei of the nutritive cells are irregular in shape and many of them possess amœboid processes. In sections of some of the nutritive cells two or more nuclei are oftentimes found, and this might easily mislead one into concluding that amitosis occurs amongst them.

4. The resting primary spermatocyte undergoes a growth period, during which thin leptotene threads are produced through the fragmentation of the chromatin. No continuous spireme is formed as the leptotene threads appear distinct and separate.

5. During the growth period pairs of leptotene threads migrate to the synaptic pole of the cell, become arranged in parallel fashion and soon fuse parasynaptically.

6. During the synizesis stage of the growth period a pair of densely staining chromatoid bodies make their appearance in the cytoplasm. These are surrounded by clear areas and may have originated from some of the chromatoid masses found within the cytoplasm of some of the earlier stages in the spermatogenesis.

7. The first spermatocyte division is reductional. In the metaphase stage the chromosomes line up as dumb-bells, which are composed of pairs of bivalents. The chromatoid bodies pass undivided to opposite poles of the cell.

8. A polar view of the metaphase stage of the reduction division reveals sixty chromosomes distributed throughout the entire plane of the equator.

9. The division of the primary spermatocytes results in secondary spermatocytes, each of which contains a chromatoid body.

10. The second spermatocyte division is equational and immediately follows the reduction division. A polar view of the metaphase stage of the equational division reveals sixty chromosomes which are about half the size of those found during the reduction division.

11. The chromatoid body passes undivided to one pole during the division of the secondary spermatocyte, resulting in the formation of two classes of spermatids, one of which contains the chromatoid body, while the other is without such a structure.

12. The chromatoid body is soon expelled from the spermatids which contain it, thus making all the spermatids alike in structure and appearance.

13. The nucleus of the spermatid loses its large quantity of intensely staining chromatin, while at the same time a mitochondria-like mass makes its appearance in the cytoplasm. Also one or two vacuoles are formed in the cytoplasm.

14. As the transformations go on the nucleus becomes elliptical and wanders to one pole of the cell. The vacuoles fuse into a single large vacuole which then takes a position at the opposite pole of the cell. The mitochondria-like mass wanders in between these two structures, becomes ring-like, and within its center and above the karyosome-like body of the nucleus, the centrosome becomes stationed. Soon a second vacuole makes its appearance at the distal end of the first one.

15. The two vacuoles gradually transform into the first and second vesicles. The centrosome and karyosome-like body of the nucleus become fused into the central body, which runs through the middle of the second vesicle, while the nucleus and mitochondria-like ring unite into a nucleus-mitochondrial cup from which the rays of the spermatozoön are produced.

16. The mature spermatozoa are oval bodies tightly packed within membranous spermatophores.

17. When the mature spermatozoa are surrounded with salt

solutions possessing a lower osmotic pressure than either the crab's body fluids or sea water, they undergo an interesting explosion in which the vesicles and the central body are completely everted, while at the same time the nuclear-mitochondrial cup rounds out into a spherical structure.

8. BIBLIOGRAPHY.

Binford, R.

'13 The Germ Cells and the Process of Fertilization in the Crab, *Menippe mercenaria*. Jour. Morph., Vol. 24, No. 2, pp. 147–202.

Carnoy, J. B.

'85 La Cytodièrése chez les Arthropodes. La Cellule, Vol. 1, pp. 189–440.

Cowdry, E. V.

'16 The General Functional Significance of Mitochondria. Am. Jour. Anat., Vol. 19, No. 3, pp. 423–446.

Fasten, N.

'14 Spermatogenesis of the American Crayfish, *Cambarus virilis* and *Cambarus immunis*(?), with Special Reference to Synapsis and the Chromatoid Bodies. Jour. Morph., Vol. 25, No. 4, pp. 587–649.

'15 The Male Reproductive Organs of Some Common Crabs of Puget Sound. Puget Sound Marine Sta. Pub., Vol. 1, No. 7, pp. 35–41.

'17 Male Reproductive Organs of Decapoda, with Special Reference to Puget Sound Forms. Puget Sound Marine Sta. Pub., Vol. 1, No. 26, pp. 285–307.

Gilson, G.

'86 Étude comparée de la spermatogénèse chez les Arthropodes. La Cellule, Vol. 2, pp. 83–239.

Grobben, C.

'78 Beiträge zur Kenntniss der männlichen Geschlechtsorgane der Dekapoden. Arb. aus dem Zool. Inst. der Univ. Wien und der Zool. Station in Triest, Vol. 1, pp. 57–150.

Herrmann, G.

'90 Notes sur la structure et le développement des spermatozoides chez les Décapodes. Bull. Sc. de la France et de la Belgique, Vol. 22, pp. 1–59.

Keppen, N.

'06 De la spermatogénèse chez *l'Astacus fluviatilis*. Mem. de la Soc. des Natural. de Kiew, Vol. 20, No. 2, pp. 231–305.

Koltzoff, N. K.

'06 Studien über die Gestalt der Zelle. I. Untersuchungen über die Spermien der Dekapoden, als Einleitung in das Problem der Zellengestalt. Arch. f. mikr. Anat. und Entw., Vol. 67, pp. 364–572.

Kornhauser, S. I.

'15 A Cytological Study of the Semi-Parasitic Copepod, *Hersilia apodiformis* (Phil.), with Some General Considerations of Copepod Chromosomes. Arch. f. Zellf., Vol. 13, pp. 339–445.

Lerat, P.

'05 Les phénomènes de maturation dans l'ovogénèse et la spermatogénèse du *Cyclops strenuus*. La Cellule, Vol. 22, pp. 163–198.

McClendon, J. F.

'10 Further Studies on the Gametogenesis of *Pandarus sinuatus*, Say. Arch. f. Zellf., Vol. 5, pp. 229–234.

Matschek, H.

'09 Zur Kenntniss der eireifung und eiablage bei den Copepoden. Zool. Anz., Vol. 34, pp. 42–54.

Montgomery, T. H.

'11 The Spermatogenesis of an Hemipteron, Euschistus. Jour. Morph., Vol. 22, No. 3, pp. 731–817.

Prowazek, S.

'02 Ein Beitrag zur Krebsspermatogenese. Zeitschr. wiss. Zool., Vol. 71, pp. 445–456.

Schreiner, A. and K. E.

'04 Die Reifungsteilungen bei den Wirbeltieren, Ein Beitrag zur Frage nach den Chromatinreduction. Anat. Anz., Vol. 24, pp. 561–578.

'06a Neue Studien über Chromatinreifung der Geschlechtzellen. I. Reifung der männlichen Geschlechtzellen von *Tomopteris onisciformis* Eschscholtz. Arch. de Biol., Vol. 22, pp. 1–66.

'06b II. Reifung der männlichen Geschlechtzellen von *Salamandra maculosa*, *Spinax niger* und *Myxine glutinosa*. Arch. de Biol., Vol. 22, pp. 419–492.

'07 Neue Studien über Chromatinreifung der Geschlechtzellen. IV. Die Reifung der Geschlechtzellen von *Enteroxenos östergreni* Bonn. Videnskabs-Selskab Skrifter; I. Math.-Naturv. Klasse, No. 2, pp. 1–25.

'08 Gibt es eine parallele Konjugation der Chromosomen? Erwiderung an die Herren Fick, Goldschmidt und Meves. Videnskabs-Selskab Skrifter: I. Math-Naturv. Klasse, No. 4, pp. 1–31.

St. George, La Valette

'92 Über die Zwitterbildung beim Flusskrebs. Arch. f. mikr. Anat., Vol. 39, pp. 504–524.

Von Winniwarter, H.

'00 Récherches sur l'ovogénèse et l'organogénèse de l'ovaire des Mammifères (lapin et homme). Arch. de Biol., Vol. 17, pp. 33–200.

Wenrich, D. H.

'16 The Spermatogenesis of *Phrynotettix magnus* with Special Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zoöl., Vol. 60, No. 3, pp. 55–134.

Wilson, E. B.

'12 Studies on Chromosomes. VIII. Observations on the Maturation Phenomena in Certain Hemiptera and Other Forms, with Considerations of Synapsis and Reduction. Jour. Exp. Zoöl., Vol. 13, pp. 345–431.

'13 A Chromatoid Body Simulating an Accessory Chromosome in Pentatoma. BIOL. BULL., Vol. 24, pp. 392–404.

9. DESCRIPTION OF PLATES.

All the illustrations in the accompanying plates were made with the aid of the camera lucida. Figs. 1–66 and 70–80 were drawn under Zeiss apochromatic objective 2 mm. Hom. Im., Comps. ocular 12,[1] at a magnification of 2,720 times. Figs. 67–69 were drawn under achromatic lenses at an approximate magnification of 1,400 times.

ABBREVIATIONS.

b = central body.
c = centrosome.
d = chromatin ring.
h = nuclear-mitochondrial cup.
k = chromatoid bodies.
m = mitochondria-like mass.
n = nucleus.
r = rays or arms of spermatozoa.
v = first or primary vacuole (first or primary vesicle).
v' = second or secondary vacuole (second or secondary vesicle).

EXPLANATION OF PLATE 1.

FIG. 1. Resting primary spermatogonial stage.

FIG. 2. Spermatogonial prophase, showing fragmentation of chromatin. Within the cytoplasm two large, heavily staining masses, probably chromatoid masses can be distinguished.

FIG. 3. Late spermatogonial prophase. Note the compact chromatin clumps within the nucleus.

FIG. 4. Metaphase, primary spermatogonium.

FIG. 5. Anaphase, primary spermatogonium.

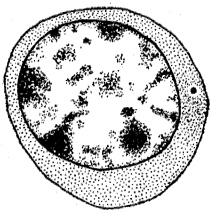
FIGS. 6 and 7. Telophase. primary spermatogonium.

FIG. 8. Resting secondary spermatogonial stage.

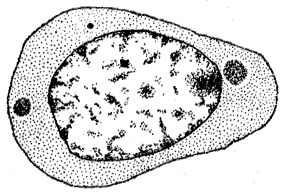
FIGS. 9 to 12. Nutritive cells. Observe the irregular nuclei and the fatty globules within the cytoplasm.

FIG. 13. Nutritive cell, in which the nucleus shows a constriction in the middle which appears to be suggestive of amitosis.

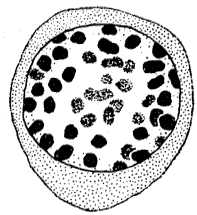
[1] I wish to express my indebtedness and gratitude to the Department of Zoölogy of the University of Wisconsin for the loan of a set of Zeiss apochromatic lenses (2 mm. oil immersion objective and compensating oculars 4, 8 and 12), which were of great service in the problem under consideration.



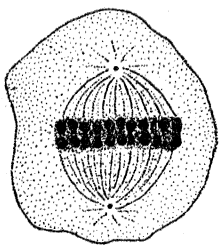
1



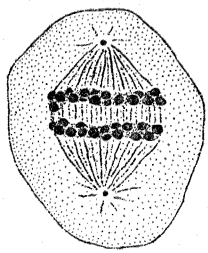
2



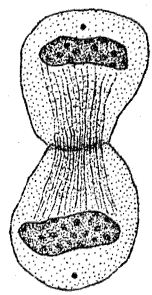
3



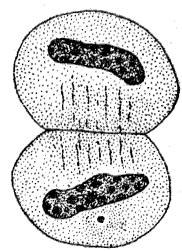
4



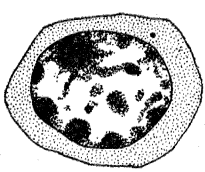
5



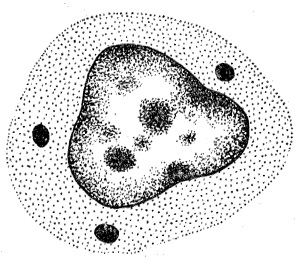
6



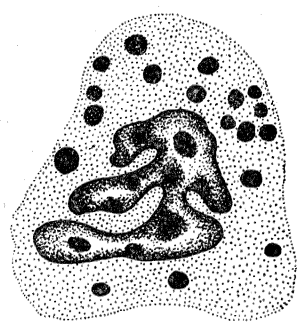
7



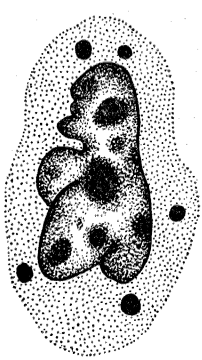
8



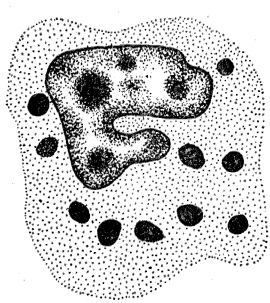
9



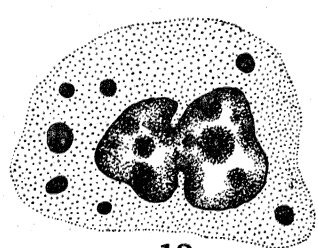
10



11



12



13

N. FASTEN, DEL.

Explanation of Plate 2.

Figs. 14 and 15. Nutritive cells, with two nuclei, suggestive of amitosis.

Figs. 16 and 17. Early prophases, primary spermatocyte stage. In Fig. 17 two darkly staining chromatoid masses can be seen within the cytoplasm.

Fig. 18. Leptotene stage.

Fig. 19. Synizesis and synapsis stage. In this stage the leptotene threads have a paired parallel arrangement at the synaptic pole of the cell. The chromatoid bodies (*k*), and the centrosome (*c*), are also visible in the cytoplasm.

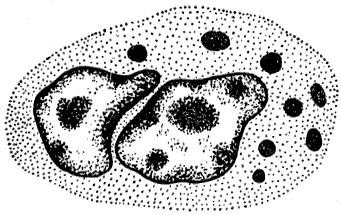
Fig. 20. Pachytene stage. The paired leptotene threads have united into thick gemini.

Figs. 21 and 22. Diplotene stage.

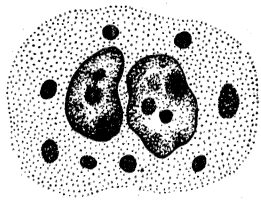
Fig. 23. Postdiplotene stage.

Fig. 24. Tetrad formation. In this stage the nuclear wall begins to break down.

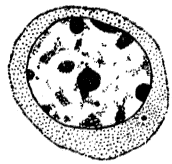
Fig. 25. Tetrads transformed into dumb-bells. The cell is entering the metaphase and the chromatoid bodies, surrounded by clear spaces are seen at opposite poles.



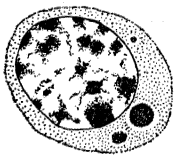
14



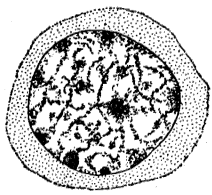
15



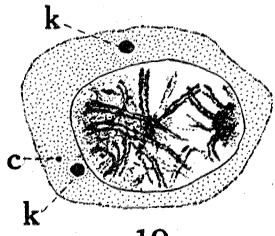
16



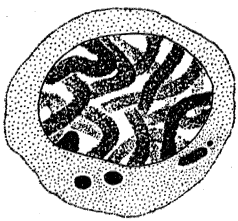
17



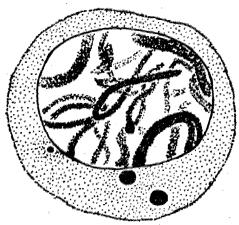
18



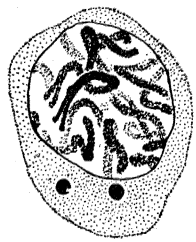
19



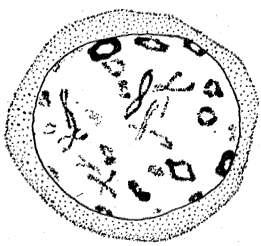
20



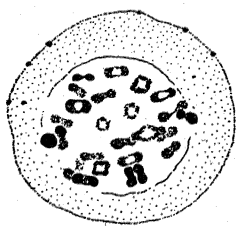
21



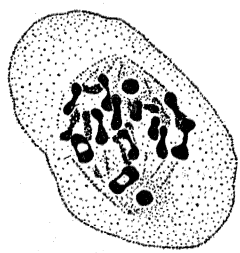
22



23



24



25

N. FASTEN, DEL.

EXPLANATION OF PLATE 3.

FIG. 26. Metaphase, primary spermatocyte showing chromatoid bodies along the spindle fibers at opposite poles.

FIGS. 27 and 28. Polar views, primary spermatocytes, showing 60 chromosomes.

FIGS. 29 to 32. Anaphase and telophase stages of primary spermatocytes showing the chromatoid bodies at opposite poles. In Fig. 32 the chromatoid bodies are observed to remain in the cytoplasm.

FIG. 33. Metaphase, secondary spermatocyte, showing a single chromatoid body at one pole.

FIGS. 34 and 35. Polar views, secondary spermatocytes, showing 60 chromosomes.

FIGS. 36 and 37. Anaphase stages, secondary spermatocytes, showing different positions which the chromatoid body may occupy in the dividing cell.

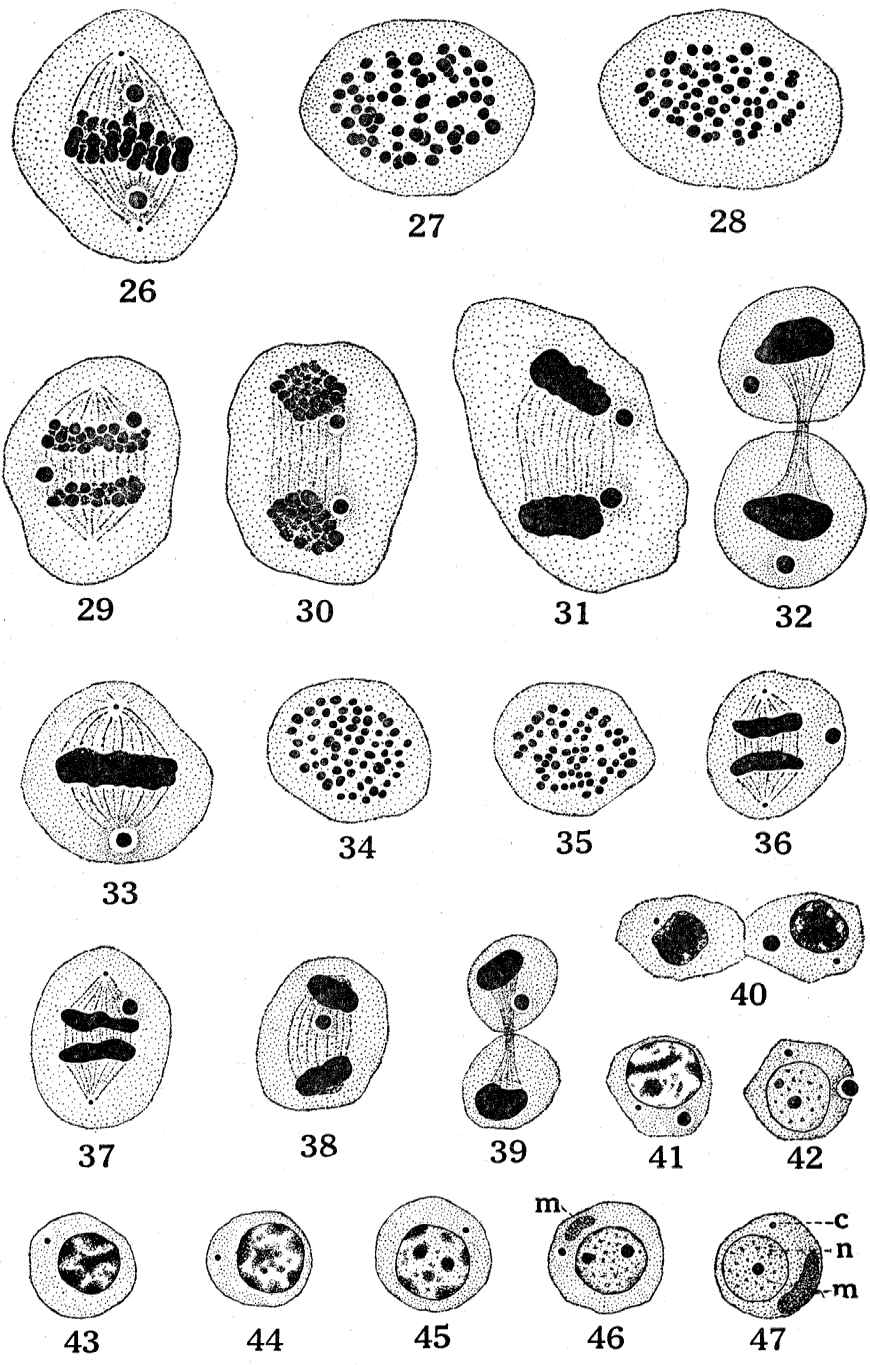
FIGS. 38 and 39. Telophase stages, secondary spermatocytes. The single chromatoid body is at one pole.

FIG. 40. The two types of spermatids formed, one without the chromatoid body, and the other containing it. The centrosomes are the minute dark granules found in the cytoplasm of both these types of spermatids.

FIG. 41. Early stages in the transforming spermatid which contains the chromatoid body.

FIG. 42. Stage in which the chromatoid body is expelled from the spermatid that contained it.

FIGS. 43 to 47. Early transformation stages of spermatids which are minus the chromatid body. Note the reduction of the chromatin and the appearance of the mitochrondria-like mass (*m*) in the cytoplasm. In Fig. 47 the single karyosome-like body occupying the center of the nucleus may be seen.



N. FASTEN, DEL.

Explanation of Plate 4.

Figs. 48 to 53. Successive stages in the transformation of the spermatid, resulting in the primary vacuole (*v*) being formed at one pole, while the nucleus (*n*) occupies the opposite pole, and in between them the mitochrondria-like mass (*m*) and the centrosome (*c*) take their position.

Fig. 54. The secondary vacuole (*v'*) makes its appearance at the distal end of the first vacuole (*v*).

Figs. 55 to 58. Stages in the transformation of the spermatid in which the primary and secondary vacuoles transform into distinct vesicles, and the central body (*b*) assumes a dumb-bell appearance.

Figs. 59 to 62. Spermatid transformations showing the hollowing out of the distal end of the central body; the formation of the chromatin-ring (*d*), and the fusion of nucleus and mitochondria-like mass into a nuclear-mitochondrial cup (*h*).

Figs. 63 and 64. Formation of the rays (*r*) of the spermatozoön.

Figs. 65 and 66. Side and bottom view of mature spermatozoa, showing details of structure.

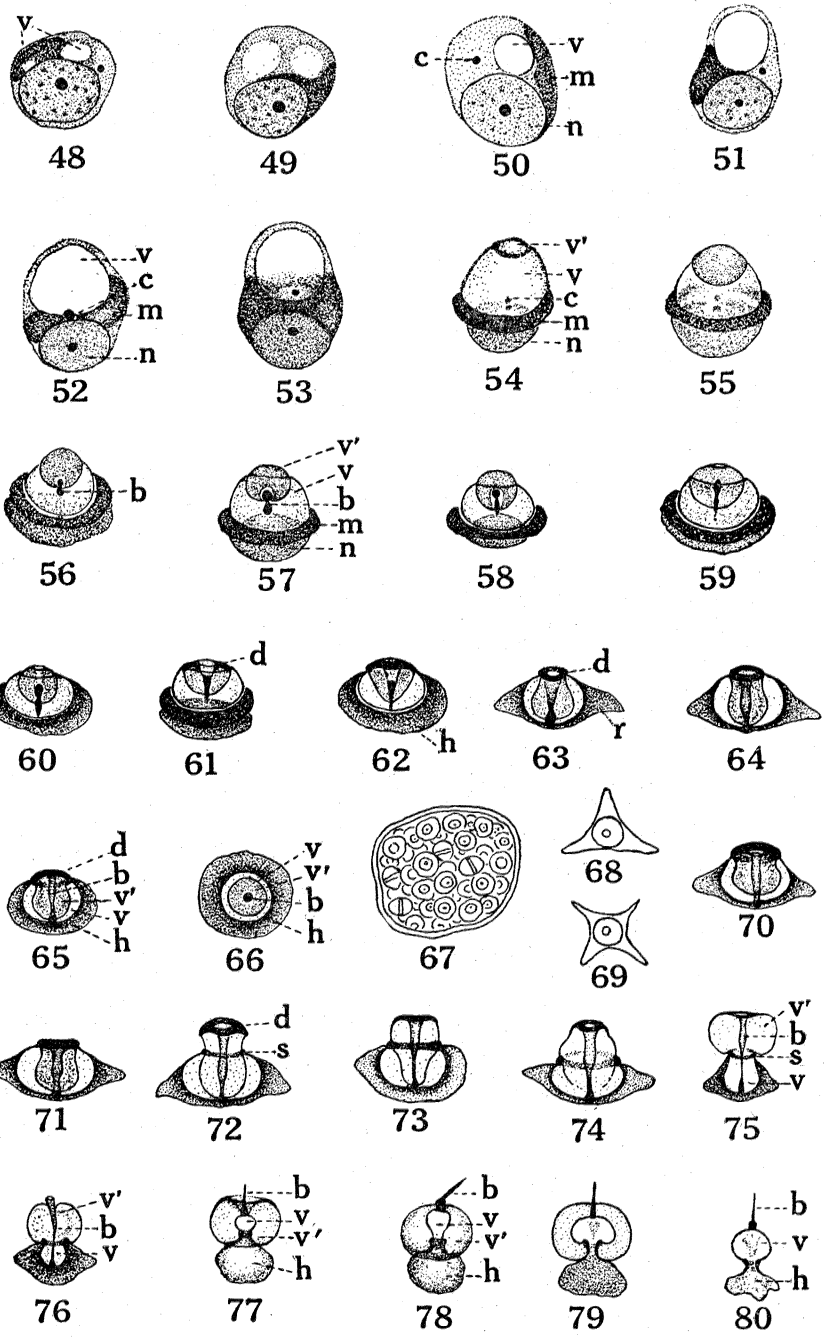
Fig. 67. Spermatophore, filled with mature spermatozoa.

Figs. 68 and 69. Three- and four-rayed types of spermatozoa, as seen suspended in the body fluid of *Cancer magister*.

Figs. 70 to 78. Successive stages in the explosion of the mature spermatozoön when surrounded with salt solutions of lower osmotic density than sea water.

Fig. 79. Spermatozoön which has exploded in distilled water.

Fig. 80. Exploded spermatozöon in which the secondary vesicle has completely disintegrated.


v
48
49
c
v
m
n
50
51
v
c
m
n
52
53
v'
v
c
m
n
54
55
b
56
v'
v
b
m
n
57
58
59
d
60
61
h
62
d
r
63
64
d
b
v'
v
h
65
v
v'
b
h
66
67
68
69
70
71
d
s
72
73
74
v'
b
s
v
75
v'
b
v
76
b
v
v'
h
77
b
v
v'
h
78
79
b
v
h
80

N. FASTEN, DEL.